

## WHAT IS CLAIMED IS:

1. A biofuel cell for generating electricity using a fuel fluid comprising:

a substrate;

a cathode supported by the substrate and capable of a reaction to reduce an oxidant in the presence of electrons to form water;

an anode supported by the substrate and capable of a reaction to oxidize the fuel fluid;

at least one of the anode and cathode including an enzyme for use in carrying out its respective reaction;

at least one of the anode and cathode being formed for flow of the fuel fluid therewithin for use in producing an electrical current.

2. The biofuel cell of claim 1, wherein the anode comprises

(a) an electron conductor;

(b) an electron mediator, the reduced form of the electron mediator being capable of releasing electrons to the electron conductor;

(c) at least one enzyme capable of reacting with the oxidized form of the electron mediator and the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator;

(d) an enzyme immobilization material capable of immobilizing and stabilizing the enzyme, the material being permeable to the fuel fluid and the electron mediator.

3. The biofuel cell of claim 1, wherein the anode comprises

(a) an electron conductor;

(b) at least one enzyme capable of reacting with an oxidized form of an electron mediator and the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator, the reduced form of the electron mediator being capable of releasing electrons to the electron conductor; and

(c) an enzyme immobilization material comprising the electron mediator, the enzyme immobilization material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the fuel fluid.

4. The biofuel cell of claim 1, wherein the anode comprises

(a) an electron conductor;

(b) an electron mediator;

5 (c) at least one enzyme capable of reacting with the oxidized form of the electron mediator and the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator;

(c) an enzyme immobilization material capable of immobilizing and stabilizing the enzyme, the material being permeable to the fuel fluid and the electron mediator; and

10 (d) an electrocatalyst adjacent the electron conductor, an oxidized form of the electrocatalyst being capable of reacting with the reduced form of the electron mediator to produce an oxidized form of the electron mediator and a reduced form of the electrocatalyst, the reduced form of the electrocatalyst being capable of releasing electrons to the electron conductor.

5. The biofuel cell of claim 1, wherein the anode comprises

(a) an electron conductor;

5 (b) at least one enzyme capable of reacting with an oxidized form of an electron mediator and the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator;

(c) an enzyme immobilization material comprising the electron mediator, the enzyme immobilization material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the fuel fluid; and

10 (d) an electrocatalyst adjacent the electron conductor, an oxidized form of the electrocatalyst being capable of reacting with the reduced form of the electron mediator to produce an oxidized form of the electron mediator and a reduced form of the electrocatalyst, the reduced form of the electrocatalyst being capable of releasing electrons to the electron conductor.

6. The biofuel cell of any one of claims 2-5 wherein the anode's electron mediator comprises nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ), flavin adenine dinucleotide (FAD), nicotinamide adenine dinucleotide phosphate (NADP), a protein, a metalloprotein, or stellacyanin.

7. The biofuel cell of any one of claims 2-3 wherein the anode's electron mediator comprises pyrroloquinoline quinone (PQQ), phenazine methosulfate, dichlorophenol indophenol, short chain ubiquinones, or potassium ferricyanide.

8. The biofuel cell of any one of claims 2-7 wherein the anode's electrocatalyst for the electron mediator comprises an azine, a conducting polymer, or an electroactive polymer.

9. The biofuel cell of claim 8 wherein the anode's electrocatalyst for the electron mediator comprises methylene green, methylene blue, luminol, nitro-fluorenone derivatives, azines, osmium phenanthroline-dione, catechol-pendant terpyridine, toluene blue, cresyl blue, nile blue, neutral red, phenazine derivatives, tionin, azure A, azure B, toluidine blue O, acetophenone, metallophthalocyanines, nile blue A, modified transition metal ligands, 1,10-phenanthroline-5,6-dione, 1,10-phenanthroline-5,6-diol, [Re(phen-dione)(CO)<sub>3</sub>Cl], [Re(phen-dione)<sub>3</sub>](PF<sub>6</sub>)<sub>2</sub>, poly(metallophthalocyanine), poly(thionine), quinones, diimines, diaminobenzenes, diaminopyridines, phenothiazine, phenoxazine, toluidine blue, brilliant cresyl blue, 3,4-dihydroxybenzaldehyde, poly(acrylic acid), poly(azure I), poly(nile blue A), poly(methylene green), poly(methylene blue), polyaniline, polypyridine, polypyrrole, polythiophene, poly(thieno[3,4-*b*]thiophene), poly(3-hexylthiophene), poly(3,4-ethylenedioxythiophene), poly(isothianaphthene), poly(3,4-ethylenedioxythiophene), poly(difluoroacetylene), poly(4-dicyanomethylene-4H-cyclopenta[2,1-*b*;3,4-*b'*]dithiophene), poly(3-(4-fluorophenyl)thiophene), poly(neutral red), a protein, a metalloprotein, or stellacyanin.

10. The biofuel cell of any one of claims 2-9 wherein the enzyme comprises an oxidoreductase.

11. The biofuel cell of claim 10 wherein the oxidoreductase comprises an oxidoreductase that acts on the CH-OH group or CH-NH group, a dehydrogenase, alcohol dehydrogenase, aldehyde dehydrogenase, formate dehydrogenase,

formaldehyde dehydrogenase, glucose dehydrogenase, glucose oxidase, lactic  
5 dehydrogenase, lactose dehydrogenase, pyruvate dehydrogenase, or a  
PQQ-dependent dehydrogenase.

12. The biofuel cell of any one of claims 1-11, wherein the cathode comprises  
(a) an electron conductor;

(b) at least one enzyme capable of reacting with a reduced form of an electron  
mediator and an oxidant to produce an oxidized form of the electron mediator and  
5 water; and

(c) an enzyme immobilization material comprising the electron mediator and an  
electrocatalyst, the enzyme immobilization material being capable of immobilizing and  
stabilizing the enzyme, the material being permeable to the oxidant, an oxidized form of  
the electrocatalyst being capable of gaining electrons from the electron conductor to  
10 produce a reduced form of the electrocatalyst that is capable of reacting with an  
oxidized form of the electron mediator to produce a reduced form of the electron  
mediator and an oxidized form of the electrocatalyst.

13. The biofuel cell of any one of claims 1-11, wherein the cathode comprises  
(a) an electron conductor;

(b) at least one enzyme capable of reacting with a reduced form of an electron  
mediator and an oxidant to produce an oxidized form of the electron mediator and  
5 water; and

(c) an enzyme immobilization material comprising an electrocatalyst, the enzyme  
immobilization material being capable of immobilizing and stabilizing the enzyme, the  
material being permeable to the oxidant, an oxidized form of the electrocatalyst being  
capable of gaining electrons from the electron conductor to produce a reduced form of  
10 the electrocatalyst which is capable of reacting with an oxidized form of the electron  
mediator to produce a reduced form of the electron mediator and an oxidized form of  
the electrocatalyst.

14. The biofuel cell of any one of claims 1-11, wherein the cathode comprises  
(a) an electron conductor;

(b) at least one enzyme capable of reacting with a reduced form of an electron mediator and an oxidant to produce an oxidized form of the electron mediator and water; and

(c) an enzyme immobilization material, the enzyme immobilization material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the oxidant, an oxidized form of the electron mediator being capable of gaining electrons from the electron conductor to produce a reduced form of the electron mediator.

15. The biofuel cell of any one of claims 1-11, wherein the cathode comprises

(a) an electron conductor;

(b) at least one enzyme capable of reacting with a reduced form of an electron mediator and an oxidant to produce an oxidized form of the electron mediator and water; and

(c) an enzyme immobilization material comprising the electron mediator, the enzyme immobilization material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the oxidant, an oxidized form of the electron mediator being capable of gaining electrons from the electron conductor to produce a reduced form of the electron mediator.

16. The biofuel cell of any one of claims 12-15 wherein the cathode's enzyme comprises an oxidoreductase.

17. The biofuel cell of claim 16 wherein the oxidoreductase comprises a laccase, an oxidase, a glucose oxidase, an alcohol-based oxidase, a cholesterol-based oxidase, an oxygen oxidoreductase, or a bilirubin oxidase.

18. The biofuel cell of any one of claims 12-17 wherein the cathode's enzyme has an optimum activity at a pH between 6.5 and 7.5.

19. The biofuel cell of any one of claims 12-13 or 16-18 wherein the cathode's electrocatalyst is an organometallic cation with standard reduction potentials greater than about +0.4 volts.



20. The biofuel cell of claim 19 wherein the organometallic cation comprises a transition metal, osmium, ruthenium, iron, nickel, rhodium, rhenium, or cobalt.

21. The biofuel cell of claim 20 wherein the organometallic cation comprises an organic aromatic ligand.

22. The biofuel cell of claim 21 wherein the large organic aromatic ligand comprises a derivative of 1,10-phenanthroline; a derivative of 2,2'-bipyridine; or a derivative of 2,2',2''-terpyridines.

23. The biofuel cell of any one of claims 12-13 or 16-22, wherein the reduced form of the electrocatalyst comprises  $\text{Ru(phenanthroline)}_3^{+2}$ ,  $\text{Fe(phenanthroline)}_3^{+2}$ ,  $\text{Ru(bipyridine)}_3^{+2}$ ,  $\text{Os(bipyridine)}_3^{+2}$ , or  $\text{Os(terpyridines)}_3^{+2}$ .

24. The biofuel cell of any one of claims 12-13 or 16-23, wherein the oxidized form of the electrocatalyst comprises  $\text{Ru(phenanthroline)}_3^{+3}$ ,  $\text{Fe(phenanthroline)}_3^{+3}$ ,  $\text{Ru(bipyridine)}_3^{+3}$ ,  $\text{Os(bipyridine)}_3^{+3}$ , or  $\text{Os(terpyridines)}_3^{+3}$ .

25. The biofuel cell of any one of claims 12-13 or 16-24, wherein the cathode's electrocatalyst is present in a concentration between about 100 mM and about 3 M.

26. The biofuel cell of any one of claims 12-13 or 16-24, wherein the cathode's electrocatalyst is present in a concentration between about 250 mM and about 2.25 M.

27. The biofuel cell of any one of claims 12-13 or 16-24, wherein the electrocatalyst is present in a concentration between about 500 mM and about 2 M.

28. The biofuel cell of any one of claims 12-13 or 16-24, wherein the electrocatalyst is present in a concentration between about 1.0 M and about 1.5 M.

29. The biofuel cell of any one of claims 12-28 wherein the oxidized form of the electron mediator comprises bilirubin, a sterol, a sugar, or a fatty acid.

30. The biofuel cell of any one of claims 12-28 wherein the oxidized form of the electron mediator comprises bilirubin and the reduced form of the electron mediator comprises biliverdin.

31. The biofuel cell of any one of claims 1-30 wherein the fuel fluid comprises hydrogen, ammonia, an alcohol, methanol, ethanol, propanol, isobutanol, butanol, isopropanol, an allyl alcohol, an aryl alcohol, glycerol, propanediol, mannitol, glucuronate, aldehyde, a carbohydrate, glucose, glucose-1, D-glucose, L-glucose, 5 glucose-6-phosphate, lactate, lactate-6-phosphate, D-lactate, L-lactate, fructose, galactose-1, galactose, aldose, sorbose, mannose, glycerate, coenzyme A, acetyl Co-A, malate, isocitrate, formaldehyde, acetaldehyde, acetate, citrate, L-gluconate, beta-hydroxysteroid, alpha-hydroxysteroid, lactaldehyde, testosterone, gluconate, a fatty acid, a lipid, phosphoglycerate, retinal, estradiol, cyclopentanol, hexadecanol, a 10 long-chain alcohol, coniferyl-alcohol, cinnamyl-alcohol, formate, a long-chain aldehyde, pyruvate, butanal, acyl-CoA, a steroid, an amino acid, flavin, NADH, NADH<sub>2</sub>, NADPH, NADPH<sub>2</sub>, a hydrocarbon, a ketone, or amine.

32. The biofuel cell of any one of claims 1-31 wherein the oxidant comprises gaseous oxygen or a peroxide compound.

33. The biofuel cell of any one of claims 1-32 wherein the fuel fluid and the oxidant are moved through the biofuel cell by at least one of electrophoretic pumping and hydrodynamic pumping.

34. The biofuel cell of any one of claims 1-32 wherein the fuel fluid and the oxidant is moved through the biofuel cell at a flow rate of between about 0.01  $\mu\text{L}/\text{min}$  and about 10  $\mu\text{L}/\text{min}$ .

35. The biofuel cell of any one of claims 1-32 wherein the fuel fluid and the oxidant is moved through the biofuel cell at a flow rate of between about 0.5  $\mu\text{L}/\text{min}$  and about 10  $\mu\text{L}/\text{min}$ .

36. The biofuel cell of any one of claims 1-32 wherein the fuel fluid and the oxidant is moved through the biofuel cell at a flow rate of between about 1  $\mu\text{L}/\text{min}$  and about 5  $\mu\text{L}/\text{min}$ .

37. The biofuel cell of any one of claims 1-32 wherein the fuel fluid is static.

38. The biofuel cell of any one of claims 2-37 wherein the enzyme immobilization material comprises a micellar or inverted micellar structure.

39. The biofuel cell of any one of claims 2-38 wherein the enzyme immobilization material comprises a modified perfluoro sulfonic acid-PTFE copolymer, the material being permeable to the fuel fluid and/or the oxidant and the electron mediator.

40. The biofuel cell of any one of claims 2-39 wherein the electron conductor comprises a carbon-based material, a metallic conductor, a semiconductor, a metal oxide, or a modified conductor.

41. The biofuel cell of any one of claims 2-40 wherein the electron conductor comprises carbon-based ink.

42. A biofuel cell for generating electricity using a fuel fluid comprising:  
a substrate;

a cathode supported by the substrate and capable of a reaction to reduce an oxidant in the presence of electrons to form water;

an anode supported by the substrate and capable of a reaction to oxidize the fuel fluid;

at least one of the anode and cathode including an enzyme for use in carrying out its respective reaction;

the cathode comprising an enzyme immobilization material comprising a micellar or inverted micellar structure.



43. A biofuel cell for generating electricity using a fuel fluid comprising:  
a substrate;

a cathode supported by the substrate and capable of a reaction to reduce an  
oxidant in the presence of electrons to form water;

5 an anode supported by the substrate and capable of a reaction to oxidize the  
fuel fluid;

at least one of the anode and cathode including an enzyme for use in carrying  
out its respective reaction;

10 at least one of the anode and cathode comprising a width less than about 200  
μm and at least one surface having an irregular, three dimensional topography capable  
of inducing convective flow of the fuel fluid and/or oxidant over said surface.

44. A biofuel cell for generating electricity using a fuel fluid comprising:  
a substrate;

a cathode supported by the substrate and capable of a reaction to reduce an  
oxidant in the presence of electrons to form water, wherein the cathode comprises:

5 (a) an electron conductor;

(b) at least one enzyme capable of reacting with a reduced form of an  
electron mediator and an oxidant to produce an oxidized form of the electron mediator  
and water; and

10 (c) an enzyme immobilization material comprising the electron mediator  
and an electrocatalyst, the enzyme immobilization material being capable of  
immobilizing and stabilizing the enzyme, the material being permeable to the oxidant,  
an oxidized form of the electrocatalyst being capable of gaining electrons from the  
electron conductor to produce a reduced form of the electrocatalyst that is capable of  
reacting with an oxidized form of the electron mediator to produce a reduced form of  
15 the electron mediator and an oxidized form of the electrocatalyst;

an anode supported by the substrate and capable of a reaction to oxidize the  
fuel fluid, wherein the anode comprises:

(a) an electron conductor;

(b) at least one enzyme capable of reacting with an oxidized form of an electron mediator and the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator;

(c) an enzyme immobilization material comprising the electron mediator, the enzyme immobilization material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the fuel fluid; and

(d) an electrocatalyst adjacent the electron conductor, an oxidized form of the electrocatalyst being capable of reacting with the reduced form of the electron mediator to produce an oxidized form of the electron mediator and a reduced form of the electrocatalyst, the reduced form of the electrocatalyst being capable of releasing electrons to the electron conductor;

at least one of the anode and cathode being formed for flow of the fuel fluid therewithin for use in producing an electrical current;

at least one of the anode's and cathode's enzyme immobilization material comprising a micellar or inverted micellar structure; and

at least one of the anode and cathode having a width less than about 200  $\mu\text{m}$ .

45. An electrode for use in the biofuel cell of any of claims 1-44, the electrode comprising an electron conductor having a width less than about 200  $\mu\text{m}$  and at least one surface having an irregular, three dimensional topography capable of inducing convective flow of the fuel fluid over said surface.

46. The electrode of claim 45, wherein the electrode has a width of between about 1  $\mu\text{m}$  and 200  $\mu\text{m}$ .

47. The electrode of claim 45, wherein the electrode has a width of between about 10  $\mu\text{m}$  and 200  $\mu\text{m}$ .

48. The electrode of claim 45, wherein the electrode has a width of between about 10  $\mu\text{m}$  and 50  $\mu\text{m}$ .

49. The electrode of claim 45, wherein the electrode has a width of between about 10  $\mu\text{m}$  and 20  $\mu\text{m}$ .

50. The electrode of claim 45, wherein the electrode comprises a carbon source.

51. The electrode of claim 45, wherein the electrode comprises a carbon-based ink.

52. The electrode of claim 45, wherein the electrode is formed for flow of the fuel fluid therewithin.

53. An electrode comprising an electron conductor having an effective surface area that is at least 1.5 times greater than its geometric surface area, wherein one dimension of the electrode is less than 100  $\mu\text{m}$ .

54. The electrode of claim 53 wherein the effective surface area is between about 1.5 times and 6 times greater than the geometric surface area.

55. A biocathode comprising:

(a) an electrode of any one of claims 45-54;

(b) at least one enzyme capable of reacting with a reduced form of an electron mediator and an oxidant to produce an oxidized form of the electron mediator and water; and

(c) an enzyme immobilization material comprising the electron mediator and an electrocatalyst, the enzyme immobilization material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the oxidant, an oxidized form of the electrocatalyst being capable of gaining electrons from the electron conductor to produce a reduced form of the electrocatalyst which is capable of reacting with an oxidized form of the electron mediator to produce a reduced form of the electron mediator and an oxidized form of the electrocatalyst.

56. A biocathode comprising:

(a) an electrode of any one of claims 45-54;

(b) at least one enzyme capable of reacting with a reduced form of an electron mediator and an oxidant to produce an oxidized form of the electron mediator and water; and

(c) an enzyme immobilization material comprising an electrocatalyst, the enzyme immobilization material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the oxidant, an oxidized form of the electrocatalyst being capable of gaining electrons from the electron conductor to produce a reduced form of the electrocatalyst which is capable of reacting with an oxidized form of the electron mediator to produce a reduced form of the electron mediator and an oxidized form of the electrocatalyst.

57. A bioanode comprising:

(a) an electrode of any one of claims 45-54;

(b) at least one enzyme capable of reacting with the oxidized form of the electron mediator and the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator, the reduced form of the electron mediator being capable of releasing electrons to the electron conductor;

(c) an enzyme immobilization material capable of immobilizing and stabilizing the enzyme, the material being permeable to the fuel fluid and the electron mediator.

58. A bioanode comprising:

(a) an electrode of any one of claims 45-54;

(b) at least one enzyme capable of reacting with an oxidized form of an electron mediator and the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator, the reduced form of the electron mediator being capable of releasing electrons to the electron conductor; and

(c) an enzyme immobilization material comprising the electron mediator, the enzyme immobilization material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the fuel fluid.

59. A bioanode comprising:

(a) an electrode of any one of claims 45-54;

(b) at least one enzyme capable of reacting with the oxidized form of the electron mediator and the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator;

(c) an enzyme immobilization material capable of immobilizing and stabilizing the enzyme, the material being permeable to the fuel fluid and the electron mediator; and

(d) an electrocatalyst adjacent the electron conductor, an oxidized form of the electrocatalyst being capable of reacting with the reduced form of the electron mediator to produce an oxidized form of the electron mediator and a reduced form of the electrocatalyst, the reduced form of the electrocatalyst being capable of releasing electrons to the electron conductor.

60. A bioanode comprising:

(a) an electrode of any one of claims 45-54;

(b) at least one enzyme capable of reacting with an oxidized form of an electron mediator and the fuel fluid to produce an oxidized form of the fuel fluid and a reduced form of the electron mediator;

(c) an enzyme immobilization material comprising the electron mediator, the enzyme immobilization material being capable of immobilizing and stabilizing the enzyme, the material being permeable to the fuel fluid; and

(d) an electrocatalyst adjacent the electron conductor, an oxidized form of the electrocatalyst being capable of reacting with the reduced form of the electron mediator to produce an oxidized form of the electron mediator and a reduced form of the electrocatalyst, the reduced form of the electrocatalyst being capable of releasing electrons to the electron conductor.

61. A method for forming an electrode for use in a biofuel cell, the method comprising

forming at least one electrical connector on a substrate;

forming at least one microchannel in a non-conductive casting mold comprised of a material that will not passivate the electrode and can be reversibly sealed to the substrate;

adhering the casting mold to the substrate;

flowing an electron conductor solution through the microchannels; and curing the electron conductor solution to form the electrode.

62. The method of claim 61, wherein the microchannels in the casting mold are formed using soft lithography.

63. The method of claim 61, wherein the casting mold is selected from the group consisting of silicon, glass, polymers, poly(dimethylsiloxane), or polycarbonate.

64. The method of claim 61, wherein the substrate is flat.

65. The method of claim 61, wherein the substrate is glass.

66. The method of claim 61, wherein the method further comprises removing the casting mold and replacing it with a gas-permeable mold comprising larger microchannels.

67. The method of claim 66, wherein the gas-permeable mold comprises silicon, glass, a polymer, poly(dimethylsiloxane), or polycarbonate.

68. The method of claim 66, wherein the microchannels in the gas-permeable mold are formed using soft lithography.

69. The method of claim 61, wherein the electron conductor solution comprises a carbon source.

70. The method of claim 61, wherein the electron conductor solution comprises a carbon-based ink.

71. The method of claim 61, wherein the electrode is cured by heating at about 75°C for about 2 hours.